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Visual Spectral Sensitivity of the Bioluminescent Deep-sea Mysid, *Gnathophausia ingens*

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Abstract. The spectral sensitivity of the deep-sea mysid, *Gnathophausia ingens* (Family Lophogastridae), was measured by electroretinography on intact specimens. High sensitivity to orange light was found. This was an unexpected result for a species whose adult members are never found above 400 m. Results of chromatic adaptation and silent substitution experiments were not compatible with either a one pigment or dual pigment visual system, making this one of the more unusual visual systems ever described.

Introduction

In this, the second of two papers on the spectral sensitivities of deep-sea crustaceans, we report on the unusual visual system of the deep-sea mysid, *Gnathophausia ingens*, a species whose adult members are found below 400 m. This robust animal survives under laboratory conditions for over two years (Childress and Price, 1978), making it an ideal candidate for electrophysiological studies. Its visual system proved to be unlike any previously described crustacean visual system, including those of the deep-sea crustaceans described in our previous paper (Frank and Case, 1988). Prolonged laboratory maintenance was not responsible for the unusual results, since specimens tested three months after capture possessed identical threshold and spectral sensitivities to those tested within 24 hours of capture.

Materials and Methods

Animal collection and maintenance

Specimens of *Gnathophausia ingens* (Family Lophigastridae) were trawled from the deep basins near San Clemente and Santa Catalina Islands, using techniques described previously (Childress and Price, 1978; Frank and Case, 1988). The light-proof collecting vessel was opened in a light-tight room, and sorting was carried out under dim red light. Specimens were placed in light-proof containers for transport back to land, where they were maintained in a 4°C cold room. Earlier studies by Childress and Price (1978) demonstrated that these mysids could survive for over two years under the proper laboratory conditions, which included maintenance in regularly changed 4°C water and a weekly feeding of salmon and shrimp. Long-term observations of specimens kept in the dark with periodic exposures to white light indicated that, although they were healthy, serious damage to their eyes had occurred. Previously bright golden eyes turned white, and tail-flip responses to red light, present in freshly caught specimens, were no longer seen. Therefore, all specimens used in this study were protected from any exposure to white light. Animals were maintained in individual quart containers placed in a light tight box, and feeding and changing of maintenance water was carried out under infrared light with the aid of an infrared image converter (FJW Industries), or eventually, under dim red light once experiments confirmed very low sensitivity to light past 650 nm. Both sexes, ranging in size from 20 to 40 mm carapace length (instars 6 through 10—Childress and Price, 1983), were used for experiments.

Optical system and recording apparatus

Electroretinograms (ERGs) were recorded using the experimental set-up described in our previous study (Frank and Case, 1988). Chromatic adaptation experiments were conducted as previously described, with a 400 nm broadband filter (Melles Griot BG12, FWHM

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= 110 nm) for violet adaptation, a 520 nm broadband filter (M.G. VG6, FWHM = 90 nm) for green adaptation, and a 570 nm short wavelength cut-off filter (M.G. OG570) for orange adaptation.

Silent substitution experiments were conducted using a modification of the methods of Forbes *et al.* (1955) and Donner and Rushton (1959). The light output from two monochromators was controlled by two electromagnetic shutters (Uniblitz) connected in such a way that when one shutter opened, the other simultaneously closed. Light from the two monochromators was conducted to the eye through the two branches of a bifurcated light guide, ensuring that upon shifting illumination from one monochromator to the other, the same receptor field was illuminated. Thus, when the two light sources are matched for equal intensity and wavelength, switching from one monochromatic source to the other should produce no visual response; *i.e.*, the substitution should be "silent." Light intensity was controlled with glass neutral density filters and a neutral density wedge.

Experimental procedure

Test flashes of 100-ms duration were used, and were repeated at one minute intervals. The response to a standard flash of set wavelength and intensity was tested every five flashes to monitor the stability of the preparation. The reciprocal of the quantum flux ($\mu W/cm^2/s$) required to elicit a set criterion response (from 20 μV to 1 mV) at each wavelength, gave the spectral sensitivity function. Absorptance curves were constructed from Dartnall nomograms (1953), using methods described by Cornwall *et al.* (1984), by an iterative process to determine the best fit to the spectral sensitivity curve.

Excellent survivorship in the experimental chamber permitted three successive chromatic adaptation experiments on the same specimens. In these experiments, lasting up to 72 hours, spectral sensitivity curves from dark-adapted and chromatically adapted eyes were measured on the first day. After recovery in the dark for ten to twelve hours, another curve from the dark-adapted eye was measured. If this post-adaptation recovery curve was the same as the pre-adaptation curve (same threshold and spectral sensitivity), a second chromatic adaptation experiment (with a different color adapting light) was performed. Subsequent experiments were conducted as long as the recovery curve and the pre-adaptation curve were the same.

Silent substitution experiments were conducted by initially illuminating the eye with monochromatic light that produced a small response (50–100 μV). Light of such a low intensity was used to ensure that long term exposure would not completely photoadapt the visual pigment(s). To demonstrate that silent substitution was possible in this species with this apparatus, light of the same wavelength from another monochromator was substituted at various time intervals, and the intensity adjusted with a neutral density wedge until no response was seen. Subsequent silent substitutions were attempted with other wavelengths of light, using a neutral density wedge to make very small intensity adjustments.

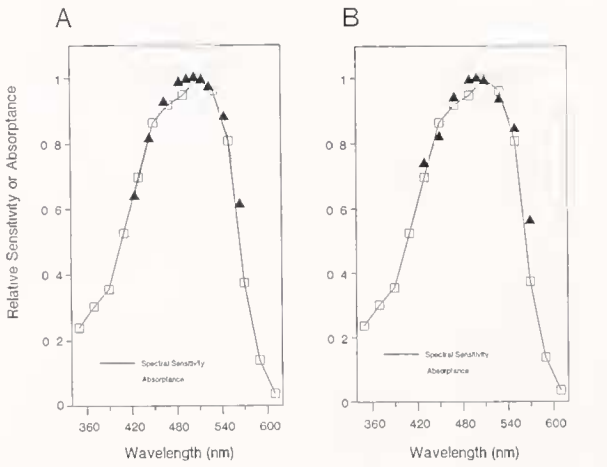

Figure 1. Solid line shows dark-adapted mean spectral sensitivity curve for *Gnathophausia ingens* (n = 25). Sensitivity is defined as the reciprocal of the relative number of quanta required to elicit a criterion response at each wavelength. Criterion responses were from 20 to 50 μV. Values from individual experiments were normalized before combining data. Standard errors are shown on the linear graph in Figure 3. The sensitivity maximum occurred around 510 nm. (A) Dotted line displays the absorptance spectrum for a hypothetical pigment with a λ_{max} of 505 nm and an O.D. of 1.2. The absorptance spectrum was calculated from the Dartnall nomogram, and plotted as the ratio of absorptance at selected wavelengths to that at 505 nm. (B) Dotted line shows the absorptance spectrum for two hypothetical pigments with λ_{max}s of 490 and 520 nm, and O.D.s of .5. Absorbance curves for the two pigments, obtained from Dartnall's template, were added together, and an absorptance curve was calculated from the result, normalized to the maximum value as above.

Regression analysis

The logarithmic spectral sensitivity values, measured at various wavelengths (every 10–20 nm from 350–610 nm) from chromatically adapted individuals, were subtracted from the values recorded from their dark-adapted eyes. The set of difference values from each animal was normalized, and the data from the same (adapting) color class were then averaged together. Linear regression lines were calculated for the mean values (Zar, 1974), and linear regression analyses were performed to determine whether the adapting lights had significant effects on the spectral sensitivities. The F-test was used to generate regressions that best fit the data. The T-test was used to determine whether the slopes of the regressions differed significantly from zero, which would indicate that the

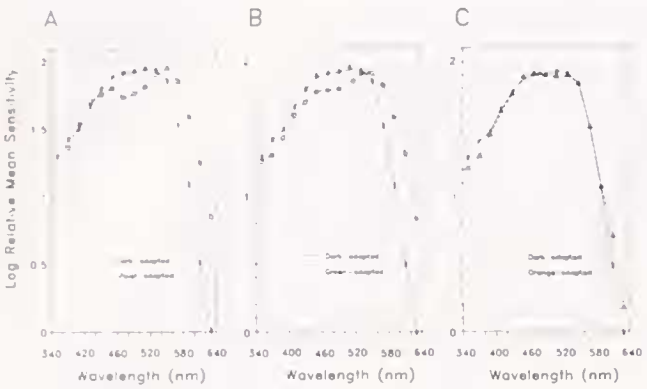

Figure 2. Effects of chromatic adaptation on the shape of the spectral sensitivity curve. Standard errors are shown in Figure 3. Values from individual experiments were normalized before combining data. (A) Average violet adapted curve (n = 11—dotted line) superimposed on the dark-adapted curve (solid line), displays two sensitivity maxima at 450 and 550 nm. (B) The effects of green adaptation (n = 7) were similar, but a short wavelength shoulder rather than a distinct peak was present. (C) Orange adaptation (n = 9) had no visible effect on the shape of the spectral sensitivity curve.

adapting light had a statistically significant effect on the shape of the spectral sensitivity curve.

Results

Spectral sensitivity

Spectral sensitivity curves were measured at criterion response amplitudes ranging from 20 μV to .5 mV. Because spectral sensitivity at all response levels was identical, only results obtained using the smallest criterion responses (from 20–80 μV) are presented, because more complete chromatically adapted curves were measured at these amplitudes.

Maximum sensitivity of dark-adapted eyes was at about 510 nm and the spectral sensitivity curve was much broader than those previously published for other crustaceans (see Fig. 2—data displayed on log scale for comparison with other published spectra). The shape of the curve is fairly well approximated by the absorptance spectrum of a single pigment present in a very high concentration (O.D. = 1.2—Fig. 1A) or by an absorptance spectrum arising from the presence of two pigments absorbing maximally at 490 and 520 nm (Fig. 1B).

Violet and green light adaptation altered the shape of the spectral sensitivity curve, but not in a manner consistent with a dual visual pigment system. Violet chromatic adaptation produced a bimodal curve with a small peak at 450 nm and a much larger 550 nm peak (Fig. 2A). Green adaptation affected the curve similarly, although the short wavelength sensitivity maximum was a shoulder rather than a peak (Fig. 2B). The effects of orange adaptation were very small (Fig. 2C); in individual animals, the curves measured under orange adaptation were identical to those measured in the dark-adapted eyes (see Fig. 5C). The chromatic adaptation results are presented on a log scale to be consistent with previously published spectral sensitivity curves on other crustaceans. However, when presented on a linear scale, the dramatic difference between the effectiveness of green and violet adaptation on altering the shape of the spectral sensitivity curve as compared with orange adaptation is much more visible (Fig. 3).

Regression analysis

Results of the linear regression analysis on the regression lines calculated for the mean difference values are shown in Figure 4. The fit to a single linear regression was poor for the data set obtained by subtracting values measured under violet adaptation from those measured in the dark-adapted eye, so these data were divided into two groups. The cut-off point for each group was chosen so that the regressions shown provided the best fit according to the F-test. The mean difference values for green adaptation were also best fit by two linear regressions, while a single regression provided the best fit to the mean difference data for orange adaptation. Both the long wavelength and short wavelength regression lines were significantly different from zero under violet adaptation ($P < .001$). Although the curve under green adaptation appears similar to that obtained under violet adaptation (Fig. 2A, B), only the long wavelength regression line (for points past 510 nm) was significantly different from zero. ($P < .001$). This indicates that green light did not have a statistically significant effect on spectral sensi-

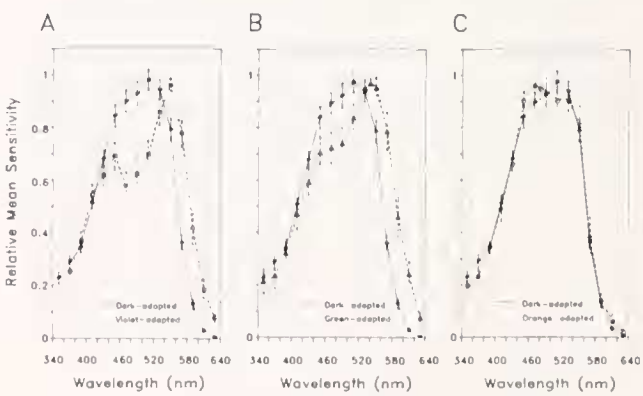

Figure 3. The effects of chromatic adaptation displayed on a linear scale, to accentuate the differences in the shapes of the chromatically adapted spectral sensitivity curves. Data are the same as those displayed in Figure 2. Bars indicate standard errors. The effects of violet and green adaptation were visibly greater and different from those of orange adaptation. A small shift in maximum sensitivity from 510 to 470 under orange adaptation is now visible.

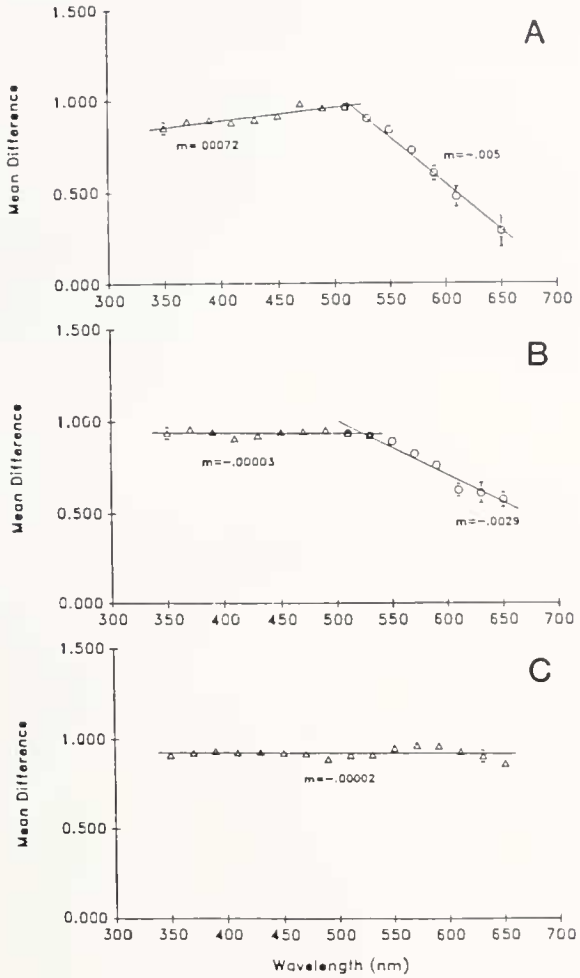

Figure 4. Statistical analysis of chromatic adaptation effects on spectral sensitivity. Each point is the mean of the difference values obtained by subtracting log light intensities required to elicit the criterion response during chromatic adaptation from those required in the dark-adapted eyes; difference values from individual animals were normalized before grouping into color classes. Standard errors are shown where they are larger than the point markers (.030). Slopes (m) of the regressions appear on each graph. (A) The mean difference values under violet chromatic adaptation (n = 11) were best fit by two regression lines. The regressions were generated using an iterative procedure in which data points were sequentially added or subtracted at 490, 510, 530, and 550 nm until the best fit was attained using the F-test. Both slopes are significantly different from zero (t = 5.89, d.f. = 7; t = 20, d.f. = 5; $P < 0.001$, both comparisons). (B) The mean difference data set for green adaptation was also best fit by two regressions. The slope of the regression from 350–530 nm is not significantly different from zero (t = .371, d.f. = 8); however, the slope of the line fitting data from 510–650 nm is (t = 11.2, d.f. = 6; $P < 0.001$). (C) The mean difference data for orange adaptation (n = 9) was best fit by one linear regression, the slope of which is not significantly different from zero (t = .110, d.f. = 14; $P \gg 0.10$).

tivity at the shorter wavelengths, but did significantly alter the shape of the spectral sensitivity curve at the longer wavelengths. The regression for the mean difference data under orange adaptation was not significantly different from zero, indicating that the shape of the spectral sensitivity curve under orange adaptation was the same as that of the dark-adapted eye.

The selective effects of the different colors were not due to intensity differences in the adapting lights. The effects were visible at the lowest intensities, and higher intensities only slightly enhanced these differences (Fig. 5). Selective effects were also not due to animal variability, as demonstrated by the results from three different chromatic adaptation experiments on one specimen (Fig. 6). This experiment also demonstrates that the results seen were not due to degenerative changes in the eye over time; complete recovery to the pre-adapted level after a sufficient dark interval can be seen (Figs. 6B, C).

Response waveforms

Analysis of the response waveforms indicates that a dual receptor system may be present, although again, the evidence is inconclusive. The ERGs were generally simple, monophasic, corneal-negative signals, characteristic of crustacean visual systems. Waveforms matched for equal amplitude in the dark-adapted eye were either similar (Fig. 7F), or, more commonly, were noticeably different only at the longest wavelengths (Fig. 7D). ERGS at the shorter wavelengths were simple in form, while at 550 to 570 nm, additional small waves appeared prior to the large wave. Occasional small positive shoulders preceding the larger negative waves were found at shorter wavelengths (Fig. 7A).

Violet, green, and orange adaptation had the same

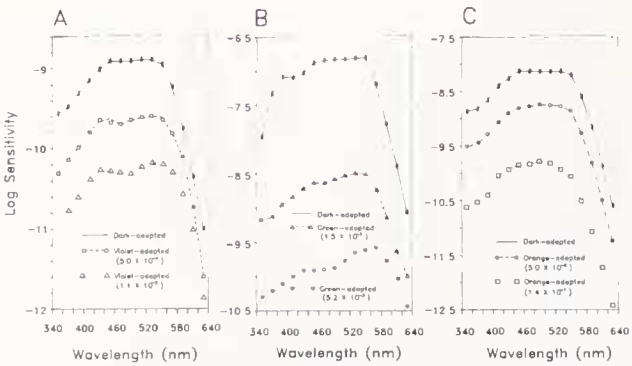

Figure 5. The effects of varying intensities of adapting lights on the spectral sensitivity function. Each graph represents data from one animal. The numbers below the plots indicate the quantum flux of the adapting light in $\mu W/cm^2/s$. (A) Violet adaptation at two different intensities produced essentially the same results. (B) The selective effects of green adaptation were more evident at the higher intensity, but were still visibly different from violet or orange adaptation at the lower intensity. (C) Selective effects of orange adaptation were not visible at either intensity, although the threshold was depressed by the same amount (1–2 log units) as under violet adaptation in A.

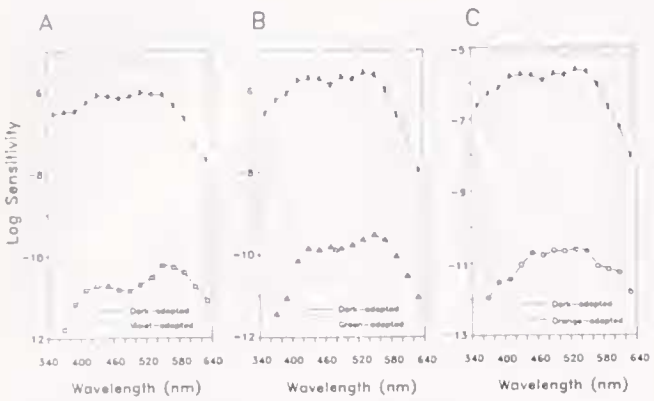

Figure 6. Selective effects of chromatic adaptation on the spectral sensitivity of one specimen. Each chromatically adapted curve is shown with the dark-adapted curve measured just prior to that chromatic adaptation experiment. The effect of the adapting light was to lower the sensitivity by approximately 4–5 log units in each experiment. The selective effects of the adapting lights are consistent with the results seen in the grouped data. (A) Blue adaptation produced two peaks at 450 and 550 nm. (B) Green adaptation similarly produced a relatively bimodal curve, with peak sensitivities at 430 and 550 nm. (C) Orange adaptation had no apparent effect on the shape of the spectral sensitivity function.

effects on the response waveforms. Under chromatic adaptation, all waveforms became biphasic, with a very small first wave (or cusp at the shortest wavelengths) followed by a second larger wave (Fig. 7B, E, G). The size of the first wave increased with increasing wavelength after 570 nm. Upon extinction of the adapting light, the response waveforms recovered to the pre-adapted state, indicating that the alteration in waveform was due to the adapting light (Fig. 7C).

Silent substitution

Substituting light from one monochromator to another did not produce a discernible dark period for *G. ingens*, since light of the same color could always be substituted without eliciting a response as long as the intensities were matched (Fig. 8A). When intensities were not properly matched, a distinct electrical signal was seen, the polarity of which depended on whether the substituting light was of a lower or a higher intensity. Silent substitution of 500 nm light for 400 nm light was always possible (Fig. 8B), indicating that the same receptor system was operating at these wavelengths. However, silent substitution was never possible between 500 and 600 nm (Fig. 8C), or 400 and 600 nm (Fig. 8D). An "on" response never completely disappeared before the "off" response became apparent. Increasing or decreasing the intensity only increased the size of the electrical signal. These results support the premise of two spectral classes of receptor cells, one dominating the responses in the blue-green, and the other operating primarily in the orange.

Discussion

Gnathophausia ingens is a deep-sea mysid whose depth distribution depends on its life history stage. The members of the size classes studied here (Instars 6–10) have a daytime depth range of 650–750 m (Childress and Price, 1978). They do not undergo a typical vertical migration, but instead disperse at night to depths between 400 and 900 m. This species possesses the typical crusta-

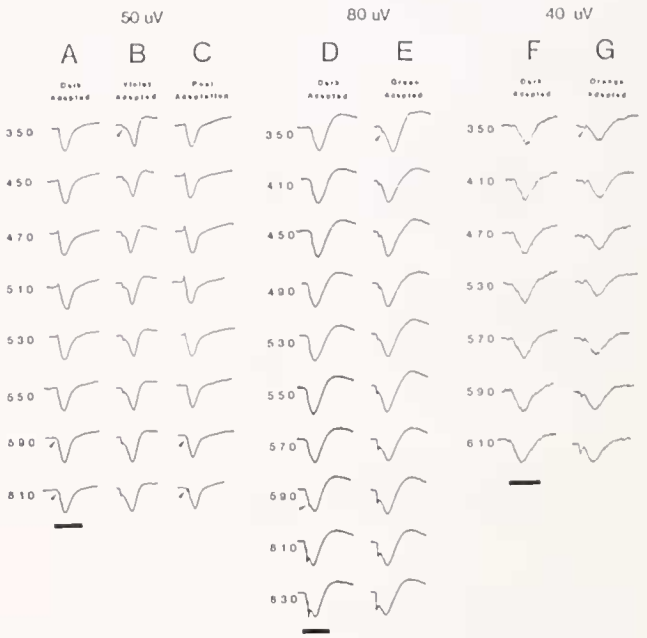

Figure 7. Response waveforms matched for equal intensity within one preparation. Data from three individuals are shown. The criterion response is shown at the top of each data set. Time bar designates 400 ms. (A) Dark-adapted waveforms were monophasic corneal-negative responses, indicated by the downward deflection, with a small corneal positive wave preceding the larger negative component at the shorter wavelengths, and a small corneal negative wave present at the longer wavelengths (arrows). (B) Violet adaptation changed all the response waveforms such that a small negative wave was present at all wavelengths (arrow), with the size of the wave increasing at the longer wavelengths. (C) Waveforms recorded two hours after extinction of the adapting light were identical to those in the dark-adapted eye. (D) The response waveforms recorded in the dark-adapted eye of another specimen were distinctly different at the longer wavelengths (arrow). The simple monophasic waveforms seen at the shorter wavelengths developed a small cusp by 590 nm, and two distinct waves were present at 610 and 630 nm. (E) The effects of a green adapting light were identical to those of the violet adapting light; the large corneal negative waves were preceded by a small corneal negative wave (arrow). The size of the small wave again increased with increasing wavelength. (F) The waveforms in this dark-adapted eye were virtually identical at all wavelengths. (G) Orange adaptation also produced the small corneal negative wave (arrow) at all wavelengths, and the small wave again increased in size at the longer wavelengths.

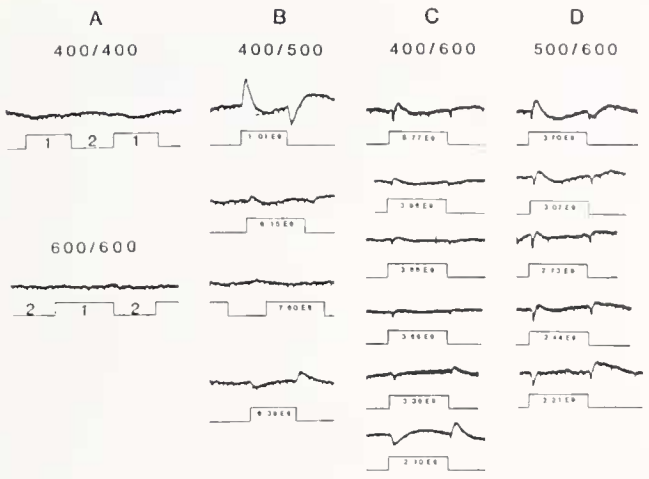

Figure 8. Results of silent substitution experiments for one specimen. The first number at the top of the graphs is the wavelength setting for Monochromator 1 (M1); the second number is for Monochromator 2 (M2). The square waves designate which monochromator was illuminating the eye for the responses seen. (A) Silent substitution was possible when M1 and M2 were set for the same wavelength, demonstrating that no discernible dark period was present during the substitution. The (1) under the square wave indicates that M1 was on at this time; when the square wave reverses polarity, light from M2 was substituted. (B) Silent substitution was also possible between 500 and 400 nm. The top figure shows the response when the intensity of the light from M1 was too high. There was a discernible "on" response at the start of the substitution, and an "off" response at the end. Responses were corneal positive in this specimen, as they were in several others, which can be attributed to the depth of the recording electrode (Konishi, 1955). The response was not a maintained "on" response for the duration of the stimulus due to the amplifier time constant. The intensity of the light from M1 is shown below the square wave pulse in photons/cm^2/s. With decreasing intensity of light, responses diminished until an intensity was reached at which no response was discernible (7.8×10^8 photons). For comparison, the last figure shows the off response when the intensity from M1 was too low and the on response upon substituting in light from M2. (C) Silent substitution was not possible between 400 and 600 nm light. Distinct on and off responses are visible in the first figure. With decreasing intensity, the on and off responses became smaller, but never disappeared. The smallest response was seen at 3.66×10^9 photons. Increasing the intensity increased the size of the on response, while decreasing the intensity produced a discernible off response. (D) Silent substitution was also not possible between 500 and 600 nm. The smallest response was at 2.73×10^9 photons, and increasing or decreasing the intensity from this value increased the size of the electrical signal.

cean spherical compound eye, but its visual physiology appears to be very unusual, and the results of this study are not entirely compatible with either a single or dual visual pigment system.

Spectral sensitivity

The absorptance curves in Figure 1 suggest two possible explanations for the unusually broad dark-adapted spectral sensitivity curve. A single pigment present in very high concentrations could give rise to such a broad spectral sensitivity curve, due to self-screening by rhodopsin. Such high concentrations of visual pigment have been found in several species of deep-sea fish (Denton and Warren, 1957) and crustaceans (Hiller-Adams *et al.*, 1988), and may increase sensitivity to light, a useful adaptation in the dimly lit deep-sea environment. However, two pigments with fairly close absorption maxima (490 and 520 nm) could also give rise to a broad spectral sensitivity curve, as demonstrated by the absorptance curve in Figure 1B.

The effects of chromatic adaptation do not support the single pigment/self-screening hypothesis. Only the response to orange adaptation is compatible with this hypothesis. The shift in maximal sensitivity is not significant, and the shape of the curve under orange adaptation is identical to the curve recorded from dark-adapted eyes in individual specimens. Under this hypothesis, the effects of green and violet adaptation should be the same as those of orange adaptation—decreasing sensitivity but not changing the position of peak sensitivity. However, violet and green light produced visible changes in the shape of the spectral sensitivity curve, and these changes were significant. Even when the adapting lights decreased the overall sensitivity by the same amount (see Fig. 6), the different colors had different effects on the spectral sensitivity. Therefore, a single visual pigment with a high optical density cannot alone explain the spectral sensitivity of *G. ingens*.

The spectral sensitivities of some shallow water lobsters, shrimp and crayfish are markedly shifted from the absorption maxima of their visual pigments (De Bruin *et al.*, 1957; Wald and Hubbard, 1957; Kennedy and Bruno, 1961; Goldsmith and Fernandez, 1968; Wald, 1968; Bruno *et al.*, 1977) and this has been attributed to selective filtering by screening pigments (Goldsmith, 1978; Cummins *et al.*, 1984). Although the eye of *Gnathophausia ingens* has not been investigated histologically, Elofsson and Hallberg (1977) and Hallberg (1977) have studied seven other species of deep-sea and shallow living mysids, and found several common characteristics. All possessed superposition eyes with a layer of red pigment cells around the proximal part of the rhabdom and below the basement membrane. In the deep-living species, the red pigment cells appeared to replace the darker retinular pigment found in other species.

Our gross histological examination of unfixed *G. ingens* eyes also revealed an abundance of red pigment cells. The eye also had a large eyeglow area, probably due to the presence of a tapetum or reflecting pigments. The eyeglow area did not change upon light adaptation, as it does in many insects and shallow living crustaceans with mobile proximal screening pigments (reviewed by Sta-

venga, 1979). This suggests that a dark proximal screening pigment is probably also absent in this species.

Due to the apparent lack of a proximal screening pigment in *Gnathophausia ingens*, the following discussion is based on the assumption that the red screening pigment is located around the proximal ends of the rhabdoms, as it is in the other deep-sea mysids lacking the proximal screening pigment (Elofsson and Hallberg, 1977; Hallberg, 1977). In this configuration, the red pigment would be in a position to filter light before it reached the visual pigment. MSP on a similar red pigment in the crab *Leptograpsus* showed that maximal absorbance was at 500 nm, with low absorbance in both the UV and red (Stowe, 1980); *i.e.*, UV and red light are not blocked by this pigment. Stowe has estimated that under strong light adaptation, when the pigment extends one third to one half way up the rhabdom, (as it does in the deep-sea mysid *Erythrops*—Hallberg, 1977), a "kink" would be seen in the spectral sensitivity curve at 380 nm. This is not the case in *G. ingens;* neither the dark-adapted or chromatically adapted eyes ever showed a violet sensitivity peak. Additionally, the long wavelength peak in *G. ingens* under violet and green adaptation always occurred between 530 and 550 nm. If a red-leaky screening pigment is responsible for the chromatically adapted spectral sensitivity (as it is in several species of muscid flies—Goldsmith, 1965), a red peak should be visible between 600 and 650 nm. Therefore, based on what is known about mysid screening pigments, the leaky screening pigment hypothesis also cannot provide an explanation for the spectral sensitivity of *G. ingens*.

In other species where there is good evidence for dual visual pigment mechanisms, the selective effects of chromatic adaptation are dramatic and undeniable under adapting lights that depressed sensitivity by 1–2 log units (Chapman and Lall, 1967; Goldsmith and Fernandez, 1968; Wald, 1968; Frank and Case, 1988). The effects of chromatic adaptation on *G. ingens* were not as distinct. Green and violet adaptation significantly changed the shape of the spectral sensitivity curve compared to orange adaptation (Fig. 4), but adapting lights that depressed sensitivity up to five log units never produced effects of the magnitude seen in other crustaceans. Additionally, we were never able to completely depress the short wavelength peak, which should be possible if a short wavelength pigment was present. Lall and Cronin (1987) describe a similar situation in the purple land crab (*Gecarcinus lateralis*). The spectral sensitivity of this species was much broader than the absorption maximum of a single visual pigment, but chromatic adaptation with different colors did not have pronounced selective effects. They suggest that this alone does not preclude the possibility of two receptor classes. Receptors containing visual pigments adjacent in the spectrum (such as blue *versus* green) would be difficult to isolate with ERGs, which are gross responses from the whole eye, and this problem would be compounded if one receptor class were numerically significantly inferior to the other class, as in the blue crab *Callinectes* (Martin and Mote, 1982).

Response waveforms

The shapes of the ERG response waveforms in the dark-adapted eye and under chromatic adaptation point towards the presence of a dual receptor mechanism, but there are inconsistencies. Under the dual receptor system hypothesis, at the shorter wavelengths, the 490 nm receptor cells would dominate the ERG. At longer wavelengths, the contribution of these shorter wavelength receptors would be diminished, and the contribution of the 520 nm receptors would become evident. The differences in response waveforms to long wavelength light (570–630 nm) and shorter wavelengths in the dark-adapted eye of *G. ingens* are consistent with this hypothesis (Fig. 7). Responses to shorter wavelengths were simple, monophasic corneal negative (downward) waveforms. At the longer wavelengths, the waveforms became more complex, with a very small negative wave preceding a much larger one. The fact that these small waves were only visible at the longest wavelengths in the dark-adapted eye, and that they became larger with increasing wavelength (Fig. 7A, D), supports the premise that they are due to the contribution of the long wavelength receptor system to the ERG.

The effects of green and violet chromatic adaptation on response waveforms are also compatible with this hypothesis. Green and violet adapting lights should and did selectively diminish the contribution of short wavelength receptors to the ERG, unmasking the contribution of the long wavelength receptors. Under these adapting lights, all waveforms were composed of a small wave preceding a larger wave, and the size of the small wave increased with increasing wavelength (Fig. 7B, E). Conversely, orange adaptation should diminish the contribution of the long wavelength receptors, and eliminate the small first waves that may have been present in the dark-adapted eye. However, this is not what occurred. The effects of orange adaptation were identical to those of violet and green adaptation; a small first wave was visible at all wavelengths and increased in size at longer wavelengths (Fig. 7G). This indicates that the small first waves are not produced by long wavelength receptor cells. These effects of orange adaptation are not consistent with the dual receptor system hypothesis, but there is currently no other physiological explanation for waveform differences in crustacean ERGs.

In insects, wavelength-specific waveform differences have been attributed to differences in the size of the gan-

glionic on/off effects in the ERG due to a leaky screening pigment. If a red leaky screening pigment is present, as in several species of muscid flies, then red stimulation would stimulate more receptors than expected, producing a larger on/off effect (Goldsmith, 1965). However, the ERG recorded in crustaceans is a more purely retinal response, with no evidence for any ganglionic component (Naka and Kawabara, 1956; Burkhardt, 1962; Chapman and Lall, 1967; Goldsmith and Fernandez, 1968). The small waves also occur only at the beginning of the response, indicating that they are not due to ganglionic contributions, since the "off" response is missing. Currently, the only explanation for waveform differences in dark-adapted crustacean eyes is that two different classes of receptor cells with different response characteristics are contributing to the ERG (Chapman and Lall, 1967; Fernandez and Goldsmith, 1968; Wald, 1968).

Silent substitution

The best evidence for a dual receptor mechanism in this species comes from the silent substitution experiments. The idea behind silent substitution (as described by Forbes *et al.*, 1955, and Donner and Rushton, 1959) is that if an eye is adapted to a steady monochromatic light, and this is replaced by a light of the same color from another source, a response will be seen if the intensity difference is within a detectable range for the eye. The substitution will only be silent when the eye can no longer detect an intensity difference, provided that the act of substitution does not produce a detectable dark period. If the lights are of different colors, the result will depend on the type of receptors contributing to the visual response. If only one type of receptor cell is present, then any two lights equally absorbed by the pigment can be silently substituted. Hence, in a single visual pigment system, an intensity can be found at each wavelength at which substitution will be silent. If more than one receptor type is contributing to the response, each with its own response characteristics, then in principle, substitution cannot be silent at all wavelengths. A response will be seen due to cessation of excitation of cells already responding, and the excitation of cells (with different membrane characteristics) previously not responding.

With our experimental design, we demonstrated that silent substitution was possible if monochromatic lights of the same colors were matched for equal intensities, indicating that an instrumental dark period was not discernible during the switch. Silent substitution was also possible between 400 and 500 nm, indicating that responses to these wavelengths are dominated by the same receptor cell class. However, 400 nm and 600 nm light could not be "silently" substituted at any intensity. Similarly, the substitution of 500 for 600 nm light always produced a discernible response. These results support the hypothesis that two receptor systems are present.

Autrum and Stumpf (1953) described the presence of a red receptor in *Musca*, basing their hypothesis partly on their heterochromatic flicker results that red light always elicited a response when substituted for blue or green light, while blue and green light intensities could be adjusted to achieve silent substitutions. However, this wavelength dependence was later attributed to stimulation of different numbers of ommatidia by red *versus* green light, due to the presence of a red leaky screening pigment (Goldsmith, 1965). Goldsmith found that it was possible to produce receptor components of equal size to green and red stimulation, but the on/off components, which are ganglionic in origin and depend on the number of receptors stimulated, could never be matched. This interpretation cannot explain our results, however, because we were working with a crustacean. As stated above, the ERG in crustaceans is a more purely retinal response, with no on/off component. Without the complicating on/off component, if a red-leaky accessory pigment was present, light intensities could be found at which weak stimulation of many receptors under red light would produce the same size response as stronger stimulation of fewer receptors under green light.

Unusual effects of adapting lights on portunid crabs (Wald, 1968; Leggett, 1979) have been attributed to the presence of a single visual pigment, whose absorption is modified by different colored filters abutting different rhabdoms upon light adaptation. Our results argue against this mechanism, as the difference in waveform responses and the inability to silently substitute between 400–500 nm *versus* 600 nm cannot be explained by a single photoreceptor class with colored filters.

We are confident that these remarkable results are not consequences of the experimental procedure. The identical apparatus was used to measure the spectral sensitivity of deep-sea oplophorids (Frank and Case, 1988) and provided clear evidence for either single or double visual pigment systems in those species, comparable to published results for shallow water crustaceans. The capture and maintenance of *Gnathophausia ingens* was identical to that of the oplophorids. The only difference is that some *G. ingens* specimens had been maintained in the laboratory for up to five months. However, both the spectral sensitivity and threshold sensitivity of specimens maintained for months in the laboratory were identical to those of specimens tested within twenty-four hours of capture, eliminating laboratory maintenance as an explanation for our unusual results.

The presence of a single highly concentrated visual pigment can be readily correlated with the deep-sea habitat of this organism, as mentioned above. However, the explanation for the long wavelength shift of peak sensi-

tivity to 510 nm, and therefore enhanced sensitivity to orange light, remains unknown. The life history of *Gnathophausia* provides no answer. The size classes used in this study are never found above 400 m, and although smaller specimens are found at shallower depths, they are always deeper than 100 m, where the spectral distribution of light has already significantly narrowed towards the bluer wavelengths (Jerlov, 1968; Dartnall, 1974; Cronin, 1986).

The rational for the presence of two visual pigments, if they are indeed present, is even more difficult to conceive. Unexpected dual visual pigment systems have been found in several species of deep-sea fish (Denton *et al.*, 1970; O'Day and Fernandez, 1974; Bowmaker, Dartnall and Herring, unpub.; Partridge *et al.*, 1988), and may be present in some deep-sea crustaceans as well (Frank and Case, 1988). All these species possess photophores, and the cited authors have suggested that possession of dual visual pigments may play a role in congener recognition. However, *G. ingens* does not possess any photophores. It does emit a bioluminescent spew from the oral region with a peak spectral emission at 485 nm (Illig, 1905; Frank *et al.*, 1984). This is close to the peak sensitivity of one of its proposed visual pigments, but is also the same as most of the bioluminescence that has been measured from organisms obtained from these depths (Herring, 1976; Widder *et al.*, 1983), which would make congener recognition based on bioluminescence difficult.

In summary, various aspects of the visual physiology of *Gnathophausia ingens* support the premise of a dual receptor system, with maximum sensitivity at 490 nm and 520 nm. These are: (1) the unusually broad dark-adapted spectral sensitivity function; (2) the selective and statistically significant effects of violet and green adaptation on the shape of the spectral sensitivity curve; (3) wavelength specific differences in response waveforms in the dark-adapted eye; (4) effects of violet and green adaptation on the response waveforms; and (5) the inability to silently substitute between 400 and 600 nm light, and 500 and 600 nm light. However, other observations argue against this conclusion. These are: (1) the inability to significantly depress the 490 nm peak; (2) the insignificant effect of orange adaptation on the shape of the spectral sensitivity curve; and (3) the identical effects of violet, green, and orange adaptation on the shape of the response waveforms. We are left with the enigma of a deep-sea crustacean with unusually high sensitivity to orange light that cannot be explained by known combinations of visual and/or screening pigments.

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